IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:
Christian REITER et al.

Application No. 09/744,176
Confirmation No. 3010

Filed: 18 June 2001

For: ANTI-HEPATITIS C VIRUS ANTIBODY AND USES THEREOF

Group Art Unit: 1648

Examiner: B. LI

Atty. Docket No. 105032-991190

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DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
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Sir:

I, Dr. Med. Christian Reiter, do hereby declare and state:

1. I am an inventor of US Application No. 09/744,176.

2. To validate the properties of hmAb 503 disclosed in the above-captioned application, various studies using known methods were conducted. Some of the methods are disclosed in the above-captioned application. The following results were obtained.

3. HCV viral glycoproteins E1 and E2 are retained in the endoplasmic reticulum. Deletion of the transmembrane domain of HCV E2 was sufficient to break off the retention. Therefore the minimal region of HCV was fused to the sequences of the transmembrane and cytoplasmic domains of CD4. To control the cloning, a myc-tag was fused to the N-terminus of the

E2 portion. This resulted in a chimeric HCV E2 protein that was targeted to the cell surface (Fig 1).

4. A binding assay was established based on this cell membrane anchored E2 via a combination of vaccinia virus (vTF7-3) infection and transfection of plasmids and diagnosed cell-bound hmAb 503 antibody using FACS analysis. Identical amount of cells were counted using a control antibody directed to myc or hmAb 503 (Fig. 2). Binding of hmAb 503 was detected by FITC-labeled anti-human IgG. Non-specific binding was controlled by the use of human IgG1, lambda, the same subtype as hmAb 503. Binding of anti-myc was detected by FITC-labeled anti-mouse IgG. Non-specific binding was controlled using murine IgG1. Dead cells were detected by propidium iodide staining.

5. hmAb 503 displayed cross-reactivity to all major genotypes and subtypes (geno- and subtypes 1a, 1b, 2a, 3a, 4a; Fig. 3).

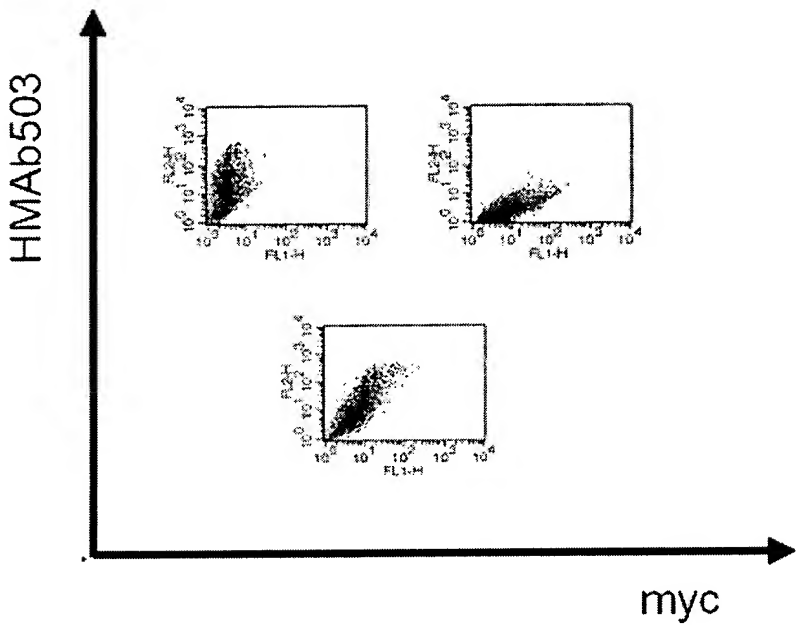
Figure 1

HCV E2 Expression Constructs

HCV-E2 Genotypes **1a, 1b, 2a, 3a, 4a**

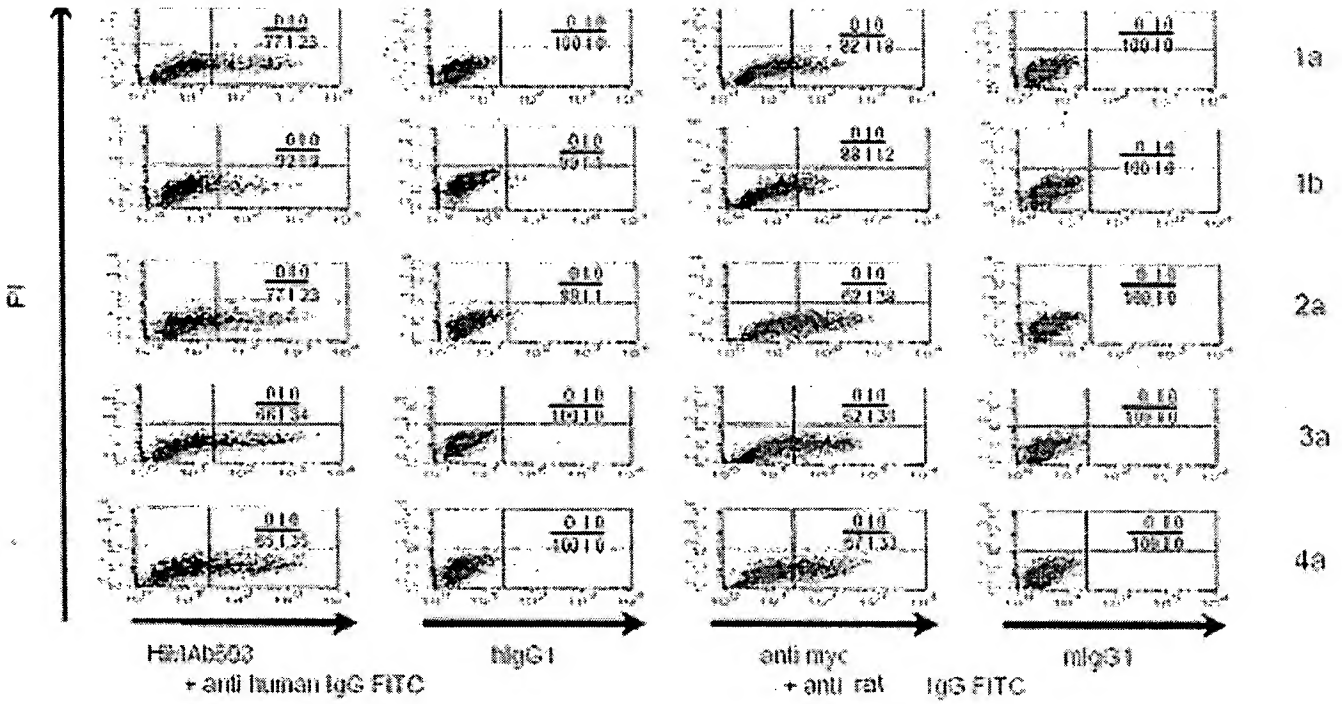
HCV-E2 expression construct containing the sequence of HCV-E2, a myc-tag and a transmembrane and intracellular domain of human CD4. Five different constructs were generated with each of genotype or subtype 1a, 1b, 2a, 3a or 4a. Cells were transfected with this construct, which targeted the myc-tag and HCV-E2 to the cell membrane.

Figure 2



HCV E2 (1a) and myc-epitope are equally expressed in E2-myc-CD4 fusion proteins. FACS staining of HeLa cells preinfected with rVV TF7-3, and transfected with pCIneoE2(1a)-myc-CD4 for 24 hours. Live cells were distinguished by use of Propidium Iodine (PI). hmAb 503 and rat anti-myc antibodies were used in single and in double staining. PI coupled rat anti-human IgG and FITC coupled mouse anti-rat IgG antibodies were used as detection antibodies.

Figure 3



HCV E2 Binding Assay. hmAb 503 recognizes all major HCV E2 genotypes (1a, 1b, 2a, 3a, 4a) and the two major subtypes 1a and 1b. FACS staining of HeLa cells pre-infected with rVV-TF7-3 and transfected with pCIneoE2-myc-CD4 (different genotypes and subtypes). Live cells were distinguished by use of PI. hmAb 503 and rat anti-myc as well as the isotype control antibodies in single stainings. FITC coupled rat anti-human and mouse anti-rat IgG antibodies were used as detection antibodies.

I hereby declare that all statements made herein are of my own knowledge and are true, and that all statements made on information and belief are believed to be true, and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued therefrom.

Further Declarant sayeth not.

_____ _____

Date Christian Reiter